

Purinergic profiling of regulatory T-cells in patients with episodic migraine

Nurkhametova D., Kudryavtsev I., Khayrutdinova O., Serebryakova M., Altunbaev R., Malm T., Giniatullin R.

Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

© 2018 Nurkhametova, Kudryavtsev, Khayrutdinova, Serebryakova, Altunbaev, Malm and Giniatullin. Objectives: Immune responses in migraine are poorly characterized, yet implicated in the disease pathogenesis. This study was carried out to characterize purinergic profiles of T-cells in patients with episodic migraine without aura (MWOA) to provide mechanistic evidence for ATP and adenosine involvement in modulation of immune regulation in migraine. Methods: Peripheral blood samples were obtained from patients with migraine (n = 16) and age-matched control subjects (n = 21). Subsets of T-cells were identified by flow cytometry based on specific membrane markers. Results: Migraine patients showed reduced total T-cell counts in the peripheral blood. Whereas the total number of CD3+CD4+, CD3+CD8+, or regulatory T lymphocytes (Treg) was not changed, the proportion of Treg CD45RO+CD62L- and CD45RO-CD62L- cells was increased. Interestingly, in migraine, less Treg cells expressed CD39 and CD73 suggesting disrupted ATP breakdown to adenosine. The negative correlations were observed between the duration of migraine and the relative number of CD73+CD39- Tregs and total number of CD73-positive CD45RO+CD62L+ Tregs. Conclusion: Obtained data indicate that T-cell populations are altered in episodic migraine and suggest the involvement of Tregs in the pathophysiology of this disorder. Reduced expression of CD39 and CD73 suggests promotion of ATP-dependent pro-inflammatory and reduction of adenosine-mediated anti-inflammatory mechanisms in migraine.

<http://dx.doi.org/10.3389/fncel.2018.00326>

Keywords

Adenosine, ATP, Migraine, Purinergic signaling, Regulatory T cells

References

- [1] Abushik P. A., Bart G., Korhonen P., Leinonen H., Giniatullina R., Sibarov D. A., (2017). Pro-nociceptive migraine mediator CGRP provides neuroprotection of sensory, cortical and cerebellar neurons via multi-kinase signaling. *Cephalalgia* 37 1373–1383. 10.1177/0333102416681588 27884929.
- [2] Ambada G. N., Ntsama C. E., Nji N. N., Ngu L. N., Sake C. N., Lissom A., (2017). Phenotypic characterization of regulatory T cells from antiretroviral-naïve HIV-1-infected people. *Immunology* 1514 405–416. 10.1111/imm.12738 28375551.
- [3] Arumugam M., Parthasarathy V., (2016). Reduction of CD4 + CD25 + regulatory t-cells in migraine: is migraine an autoimmune disorder? *J. Neuroimmunol.* 290 54–59. 10.1016/j.jneuroim.2015.11.015 26711570.

- [4] Ashina M., Bendtsen L., Jensen R., Schifter S., Olesen J., (2000). Evidence for increased plasma levels of calcitonin gene-related peptide in migraine outside of attacks. *Pain* 86 133-138. 10.1016/S0304-3959(00)00232-3.
- [5] Aspelund A., Antila S., Proulx S. T., Karlsen T. V., Karaman S., Detmar M., (2015). A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. *J. Exp. Med.* 212 991-999. 10.1084/jem.20142290 26077718.
- [6] Bashir A., Lipton R. B., Ashina S., (2013). Migraine and structural changes in the brain: a systematic review and meta-analysis. *Neurology* 81 1260-1268. 10.1212/WNL.0b013e3182a6cb32 23986301.
- [7] Boćkowski L., Sobaniec W., Żelazowska-Rutkowska B., (2009). Proinflammatory plasma cytokines in children with migraine. *Pediatr. Neurol.* 41 17-21. 10.1016/j.pediatrneurol.2009.02.001 19520268.
- [8] Bolay H., Reuter U., Dunn A., Huang Z., Boas D. A., Moskowitz M. A., (2002). Intrinsic brain activity triggers trigeminal meningeal afferents in a migraine model. *Nat. Med.* 8 136-142. 10.1038/nm0202-136 11821897.
- [9] Bono M. R., Fernández D., Flores-Santibáñez F., Roseblatt M., Sauma D., (2015). CD73 and CD39 ectonucleotidases in T cell differentiation: beyond immunosuppression. *FEBS Lett.* 589 3454-3460. 10.1016/j.febslet.2015.07.027 26226423.
- [10] Borsellino G., Kleinewietfeld M., Di Mitri D., Sternjak A., Diamantini A., Giometto R., (2007). Expression of ectonucleotidase CD39 by Foxp3 + Treg cells: hydrolysis of extracellular ATP and immune suppression. *Blood* 110 1225-1232. 10.1182/blood-2006-12-064527 17449799.
- [11] Burnstock G., (1981). Pathophysiology of migraine: a new hypothesis. *Lancet* 317 1397-1399. 10.1016/S0140-6736(81)92572-1.
- [12] Burnstock G., (2016). P2X ion channel receptors and inflammation. *Purinergic Signal.* 12 59-67. 10.1007/s11302-015-9493-0 26739702.
- [13] Cernuda-Morollón E., Martínez-Camblor P., Ramón C., Larrosa D., Serrano-Pertierra E., Pascual J., (2014). CGRP and VIP levels as predictors of efficacy of Onabotulinumtoxin type A in chronic migraine. *Headache* 54 987-995. 10.1111/head.12372 24673487.
- [14] Chen S. P., Qin T., Seidel J. L., Zheng Y., Eikermann M., Ferrari M. D., (2017). Inhibition of the P2X7-PANX1 complex suppresses spreading depolarization and neuroinflammation. *Brain* 140 1643-1656. 10.1093/brain/awx085 28430869.
- [15] Colombo B., Dalla Libera D., Comi G., (2011). Brain white matter lesions in migraine: What's the meaning? *Neurol. Sci.* 32 S37-S40. 10.1007/s10072-011-0530-7 21533710.
- [16] de Oliveira Bravo M., Carvalho J., Saldanha-Araujo F., (2016). Adenosine production: a common path for mesenchymal stem-cell and regulatory T-cell-mediated immunosuppression. *Purinergic Signal.* 12 595-609. 10.1007/s11302-016-9529-0 27557887.
- [17] Ehrentraut H., Clambey E. T., McNamee E. N., Brodsky K. S., Ehrentraut S. F., Poth J. M., (2013). CD73 + regulatory T cells contribute to adenosine-mediated resolution of acute lung injury. *FASEB J.* 27 2207-2219. 10.1096/fj.12-225201 23413361.
- [18] Ferrari D., Pizzirani C., Adinolfi E., Lemoli R. M., Curti A., Idzko M., (2006). The P2X7 receptor: a key player in IL-1 processing and release. *J. Immunol.* 176 3877-3883. 10.4049/jimmunol.176.7.3877.
- [19] Fletcher J. M., Lonergan R., Costelloe L., Kinsella K., Moran B., O'Farrelly C., (2009). CD39 + Foxp3 + regulatory T cells suppress pathogenic Th17 cells and are impaired in multiple sclerosis. *J. Immunol.* 183 7602-7610. 10.4049/jimmunol.0901881 19917691.
- [20] Giniatullin R., Nistri A., Fabbretti E., (2008). Molecular mechanisms of sensitization of pain-transducing P2X3 receptors by the migraine mediators CGRP and NGF. *Mol. Neurobiol.* 37 83-90. 10.1007/s12035-008-8020-5 18459072.
- [21] Gölöncsér F., Sperlággh B., (2014). Effect of genetic deletion and pharmacological antagonism of P2X7 receptors in a mouse animal model of migraine. *J. Headache Pain* 15:24. 10.1186/1129-2377-15-24 24885962.
- [22] Gong N., Liu J., Reynolds A. D., Gorantla S., Mosley R. L., Gendelman H. E., (2011). Brain ingress of regulatory T cells in a murine model of HIV-1 encephalitis. *J. Neuroimmunol.* 230 33-41. 10.1016/j.jneuroim.2010.08.014 20846730.
- [23] Granstein R. D., Wagner J. A., Stohl L. L., Ding W., (2015). Calcitonin gene-related peptide: key regulator of cutaneous immunity. *Acta Physiol.* 213 586-594. 10.1111/apha.12442 25534428.
- [24] Grant C. R., Liberal R., Mieli-Vergani G., Vergani D., Longhi M. S., (2015). Regulatory T-cells in autoimmune diseases: challenges, controversies and—yet—unanswered questions. *Autoimmun. Rev.* 14 105-116. 10.1016/j.autrev.2014.10.012 25449680.
- [25] Gronert A. A., Fytili P., Suneetha P. V., Kraft A. R., Brauner C., Schlue J., (2015). Comprehensive phenotyping of regulatory T cells after liver transplantation. *Liver Transpl.* 21 381-395. 10.1002/lt.24050 25451888.
- [26] Headache Classification Committee of the International Headache Society (IHS) (2018). The international classification of headache disorders, 3rd edition. *Cephalalgia* 33 629-808. 10.1177/0333102417738202 29368949.

- [27] Horenstein A. L., Chillemi A., Zini R., Quarona V., Bianchi N., Manfredini R., (2018). Cytokine-induced killer cells express CD39, CD38, CD203a, CD73 ectoenzymes and P1 adenosinergic receptors. *Front. Pharmacol.* 9:196. 10.3389/fphar.2018.00196 29731713.
- [28] Hougaard A., Amin F. M., Ashina M., (2014). Migraine and structural abnormalities in the brain. *Curr. Opin. Neurol.* 27 309–314. 10.1097/WCO.000000000000086 24751961.
- [29] Hu R., Li Y. J., Li X. H., (2016). An overview of non-neural sources of calcitonin gene-related peptide. *Curr. Med. Chem.* 23 763–773. 10.2174/0929867323666160210125416 26861004.
- [30] Huang Y., Liu Z., Cao B. B., Qiu Y. H., Peng Y. P., (2017). Treg cells protect dopaminergic neurons against MPP + neurotoxicity via CD47-SIRPA interaction. *Cell. Physiol. Biochem.* 41 1240–1254. 10.1159/000464388 28268219.
- [31] Hung A. L., Lim M., Doshi T. L., (2017). Targeting cytokines for treatment of neuropathic pain. *Scand. J. Pain* 17 287–293. 10.1016/j.sjpain.2017.08.002 29229214.
- [32] Karmakar M., Katsnelson M. A., Dubyak G. R., Pearlman E., (2016). Neutrophil P2X7 receptors mediate NLRP3 inflammasome-dependent IL-1 β secretion in response to ATP. *Nat. Commun.* 7:10555. 10.1038/ncomms10555 26877061.
- [33] Khaiboullina S., Mendelevich E., Shigapova L., Shagimardanova E., Gazizova G., Nikitin A., (2017). Cerebellar atrophy and changes in cytokines associated with the CACNA1A R583Q mutation in a russian familial hemiplegic migraine type 1 family. *Front. Cell. Neurosci.* 11:263. 10.3389/fncel.2017.00263 28900389.
- [34] Kilinc E., Guerrero-Toro C., Zakharov A., Vitale C., Gubert-Olive M., Koroleva K., (2017). Serotonergic mechanisms of trigeminal meningeal nociception: implications for migraine pain. *Neuropharmacology* 166 160–173. 10.1016/j.neuropharm.2016.28025094.
- [35] Kobie J. J., Shah P. R., Yang L., Rebhahn J. A., Fowell D. J., Mosmann T. R., (2006). T regulatory and primed uncommitted CD4 T cells express CD73, which suppresses effector CD4 T cells by converting 5'-adenosine monophosphate to adenosine. *J. Immunol.* 177 6780–6786. 10.4049/jimmunol.177.10.6780 17082591.
- [36] la Sala A., Ferrari D., Di Virgilio F., Idzko M., Norgauer J., Girolomoni G., (2003). Alerting and tuning the immune response by extracellular nucleotides. *J. Leukoc. Biol.* 73 339–343. 10.1189/jlb.0802418 12629147.
- [37] Levy D., (2009). Migraine pain, meningeal inflammation, and mast cells. *Curr. Pain Headache Rep.* 13 237–240. 10.1016/j.neuropharm.2016.12.024 28025094.
- [38] Li P., Gan Y., Sun B. L., Zhang F., Lu B., Gao Y., (2013). Adoptive regulatory T-cell therapy protects against cerebral ischemia. *Ann. Neurol.* 74 458–471. 10.1002/ana.23815 23674483.
- [39] Li P., Gao Y., Cao J., Wang W., Chen Y., Zhang G., (2015). CD39 + regulatory T cells attenuate allergic airway inflammation. *Clin. Exp. Allergy* 45 1126–1137. 10.1111/cea.12521 25728362.
- [40] Liu J., Gong N., Huang X., Reynolds A. D., Mosley R. L., Gendelman H. E., (2009). Neuromodulatory activities of CD4 + CD25 + regulatory T cells in a murine model of HIV-1-associated neurodegeneration. *J. Immunol.* 182 3855–3865. 10.4049/jimmunol.0803330 19265165.
- [41] Louveau A., Smirnov I., Keyes T. J., Eccles J. D., Rouhani S. J., Peske J. D., (2015). Structural and functional features of central nervous system lymphatic vessels. *Nature* 523 337–341. 10.1038/nature14432 26030524.
- [42] Magni G., Ceruti S., (2013). P2Y purinergic receptors: new targets for analgesic and antimigraine drugs. *Biochem. Pharmacol.* 85 466–477. 10.1016/j.bcp.2012.10.027 23146663.
- [43] Mahnke Y. D., Roederer M., (2007). Optimizing a multicolor immunophenotyping assay. *Clin. Lab. Med.* 27 469–485. 10.1016/j.cll.2007.05.002 17658403.
- [44] Mandapathil M., Hildorfer B., Szczepanski M. J., Czystowska M., Szajnik M., Ren J., (2010). Generation and accumulation of immunosuppressive adenosine by human CD4 + CD25highFOXP3 + regulatory T cells. *J. Biol. Chem.* 285 7176–7186. 10.1074/jbc.M109.047423 19858205.
- [45] Matsuda R., Kezuka T., Nishiyama C., Usui Y., Matsunaga Y., Okunuki Y., (2012). Suppression of murine experimental autoimmune optic neuritis by mature dendritic cells transfected with calcitonin gene-related peptide gene. *Invest. Ophthalmol. Vis. Sci.* 53 5475–5485. 10.1167/iovs.12-9935 22807299.
- [46] Moskowitz M. A., Cutrer F. M., (1993). Sumatriptan: a receptor-targeted treatment for migraine. *Annu. Rev. Med.* 44 145–154. 10.1146/annurev.me.44.020193.001045.
- [47] Moskowitz M. A., Reinhard J. F., Jr. Romero J., Melamed E., Pettibone D. J., (1979). Neurotransmitters and the fifth cranial nerve: is there a relation to the headache phase of migraine? *Lancet* 2 883–885. 90971.
- [48] Munno I., Marinaro M., Bassi A., Cassiano M. A., Causarano V., Centonze V., (2001). Immunological aspects in migraine: increase of IL-10 plasma levels during attack. *Headache* 41 764–767. 10.1046/j.1526-4610.2001.01140.x 11576199.
- [49] Neal E. G., Acosta S. A., Kaneko Y., Ji X., Borlongan C. V., (2018). Regulatory T-cells within bone marrow-derived stem cells actively confer immunomodulatory and neuroprotective effects against stroke. *J. Cereb. Blood Flow Metab.* 10.1177/0271678X18766172 [Epub ahead of print]. 29569981.
- [50] Olesen J., Burstein R., Ashina M., Tfelt-Hansen P., (2009). Origin of pain in migraine: evidence for peripheral sensitisation. *Lancet Neurol.* 8 679–690. 10.1016/S1474-4422(09)70090-0 19539239.

- [51] Peelen E., Damoiseaux J., Smolders J., Knippenberg S., Menheere P., Tervaert J., (2011). Th17 expansion in MS patients is counterbalanced by an expanded CD39 + regulatory T Cell population during remission but not during relapse. *J. Neuroimmunol.* 24 97–103. 10.1016/j.jneuroim.2011.09.013 22035960.
- [52] Peroutka S. J., (2005). Neurogenic inflammation and migraine: implications for the therapeutics. *Mol. Interv.* 5 304–311. 10.1124/mi.5.5.10 16249526.
- [53] Pietrobon D., Moskowitz M. A., (2013). Pathophysiology of migraine. *Annu. Rev. Physiol.* 75 365–391. 10.1146/annurev-physiol-030212-183717 23190076.
- [54] Ramachandran R., (2018). Neurogenic inflammation and its role in migraine. *Semin. Immunopathol.* 40 301–314. 10.1007/s00281-018-0676-y 29568973.
- [55] Reuter U., Bolay H., Jansen-Olesen I., Chiarugi A., Sanchez del Rio M., Letourneau R., (2001). Delayed inflammation in rat meninges: implications for migraine pathophysiology. *Brain* 124 2490–2502. 10.1093/brain/124.12.2490 11701602.
- [56] Rochlitz S., Veres T. Z., Kühne K., Prenzler F., Pilzner C., Knothe S., (2016). The neuropeptide calcitonin gene-related peptide affects allergic airway inflammation by modulating dendritic cell function. *Clin. Exp. Allergy* 41 1609–1621. 10.1111/j.1365-2222.2011.03822.x 21752117.
- [57] Rueda C. M., Jackson C. M., Chougnet C. A., (2016). Regulatory T-cell-mediated suppression of conventional T-cells and dendritic cells by different cAMP intracellular pathways. *Front. Immunol.* 7:216. 10.3389/fimmu.2016.00216 27313580.
- [58] Sakaguchi S., Miyara M., Costantino C. M., Hafler D. A., (2010). FOXP3 + regulatory T cells in the human immune system. *Nat. Rev. Immunol.* 10 490–500. 10.1038/nri2785 20559327.
- [59] Sarchielli P., Alberti A., Baldi A., Coppola F., Rossi C., Pierguidi L., (2006). Proinflammatory cytokines, adhesion molecules, and lymphocyte integrin expression in the internal jugular blood of migraine patients without aura assessed ictally. *Headache* 46 200–207. 10.1111/j.1526-4610.2006.00337.x 16492228.
- [60] Somjen G. G., (2001). Mechanisms of spreading depression and hypoxic spreading depression-like depolarization. *Physiol. Rev.* 81 1065–1096. 10.1152/physrev.2001.81.3.1065 11427692.
- [61] Strassman A. M., Raymond S. A., Burstein R., (1996). Sensitization of meningeal sensory neurons and the origin of headaches. *Nature* 384 560–564.
- [62] Szklany K., Ruiter E., Mian F., Kunze W., Bienenstock J., Forsythe P., (2016). Superior cervical ganglia neurons induce Foxp3 + regulatory T cells via calcitonin gene-related peptide. *PLoS One* 11:e0152443. 10.1371/journal.pone.0152443 27022966.
- [63] Takenaka M. C., Robson S., Quintana F. J., (2016). Regulation of the T cell response by CD39. *Trends Immunol.* 37 427–439. 10.1016/j.it.2016.04.009 27236363.
- [64] Thalakoti S., Patil V. V., Damodaram S., Vause C. V., Langford L. E., Freeman S. E., (2007). Neuron-glia signaling in trigeminal ganglion: implications for migraine pathology. *Headache* 47 1008–1023. 10.1111/j.1526-4610.2007.00854.x 17635592.
- [65] Turan H., Horasanli B., Ugur M., Arslan H., (2011). Procalcitonin levels in migraine patients. *Can. J. Neurol. Sci.* 38 124–128. 10.1017/S0317167100011161.
- [66] Vanmolkot F. H., de Hoon J. N., (2007). Increased C-reactive protein in young adult patients with migraine. *Cephalalgia* 27 843–846. 10.1111/j.1468-2982.2007.01324.x 17668468.
- [67] Vignali D. A., Collison L. W., Workman C. J., (2008). How regulatory T cells work. *Nat. Rev. Immunol.* 8 523–532. 10.1038/nri2343 18566595.
- [68] Wang F., He Q., Ren Z., Li F., Chen W., Lin X., (2015). Association of serum levels of intercellular adhesion molecule-1 and interleukin-6 with migraine. *Neurol. Sci.* 36 535–540. 10.1007/s10072-014-2010-3 25417066.
- [69] Wang L., Fan J., Chen S., Zhang Y., Curiel T. J., Zhang B., (2013). Graft-versus-host disease is enhanced by selective CD73 blockade in mice. *PLoS One* 8:e58397. 10.1371/journal.pone.0058397 23520507.
- [70] Welch K. M., Brandes A. W., Salerno L., Brandes J. L., (2006). C-reactive protein may be increased in migraine patients who present with complex clinical features. *Headache* 46 197–199. 10.1111/j.1526-4610.2006.00330.x 16492227.
- [71] Yegutkin G. G., (2008). Nucleotide- and nucleoside-converting ectoenzymes: important modulators of purinergic signalling cascade. *Biochim. Biophys. Acta* 1783 673–694. 10.1016/j.bbamcr.2008.01.024 18302942.
- [72] Yegutkin G. G., Guerrero-Toro C., Kilinc E., Koroleva K., Ishchenko Y., Abushik P., (2016). Nucleotide homeostasis and purinergic nociceptive signaling in rat meninges in migraine-like conditions. *Purinergic Signal.* 12 561–574. 10.1007/s11302-016-9521-8 27369815.
- [73] Yilmaz N., Yilmaz M., Sirin B., Yilmaztekin S., Kutlu G., (2017). The relationship between levels of plasma-soluble urokinase plasminogen activator receptor (suPAR) and presence of migraine attack and aura. *J. Recept. Signal. Transduct. Res.* 37 447–452. 10.1080/10799893.2017.1328440 28553881.
- [74] Yoshida O., Dou L., Kimura S., Yokota S., Isse K., Robson S. C., (2015). CD39 deficiency in murine liver allografts promotes inflammatory injury and immune-mediated rejection. *Transpl. Immunol.* 32 76–83. 10.1016/j.trim.2015.01.003 25661084.

- [75] Yücel M., Kotan D., Gurol Çiftçi G., Çiftçi I. H., Cikrikler H. I., (2016). Serum Levels of endocan, claudin-5 and cytokines in migraine. *Eur. Rev. Med. Pharmacol. Sci.* 20 930-936. 27010153.
- [76] Zakharov A., Koroleva K., Giniatullin R., (2016). Clustering analysis for aortic ATP-induced nociceptive firing in rat meninges. *BioNanoScience* 6 508-512. 10.1007/s12668-016-0276-z.
- [77] Zakharov A., Vitale C., Kilinc E., Koroleva K., Fayuk D., Shelukhina I., (2015). Hunting for origins of migraine pain: cluster analysis of spontaneous and capsaicin-induced firing in meningeal trigeminal nerve fibers. *Front. Cell Neurosci.* 9:287. 10.3389/fncel.2015.00287 26283923.